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<p>(54) Title: NOVEL CONJUGATES OF RGD-CONTAINING PEPTIDES AND ENDOGENOUS CARRIERS</p> <p>(57) Abstract</p> <p>Conjugates are prepared from RGD containing peptides, by combining said peptides or analog with a material providing a functionally reactive group capable of reacting with a blood component (preferably a mobile blood cell or endogenous protein). The conjugates may be administered to patients to provide antiplatelet or antiadhesion properties through the inhibition of the binding of fibrinogen to the GPIIb/IIIa receptor, and may also be used as probes for receptor activity. The administration to the patient may be made either <i>in vivo</i> or <i>ex vivo</i> and may be performed by either introducing the RDG containing peptide including the reactive functional group into the patient's vascular system or preparing such a conjugate externally and introducing that conjugate to the patient's vascular system.</p>		

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NOVEL CONJUGATES OF RGD-CONTAINING PEPTIDES AND ENDOGENOUS CARRIERS

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RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of United States Provisional Patent Application number 60/064,705 filed November 7, 1997.

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FIELD OF THE INVENTION

This invention relates to conjugates of RGD-containing peptides and endogenous carriers, particularly to RGD-containing peptides and various mobile blood components, particularly mobile endogenous proteins.

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BACKGROUND OF THE INVENTION

Pharmacologic dissolution of established thrombi has become a well accepted therapeutic procedure for many patients who develop myocardial infarction or other thrombotic occlusive diseases. However, currently available therapy has a number of important limitations. A significant percentage (25-30%) of patients with acute myocardial infarction do not experience successful coronary reperfusion, despite the use of the most potent thrombolytic agents or combinations thereof. Systemic fibrinogenolysis and consequent bleeding are encountered frequently. Further, 10-30% of patients experience acute coronary reocclusion following thrombolysis.

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The interaction between fibrinogen and GPIIb/IIIa on the surface of platelets is one of the critical steps involved in platelet

aggregation and rethrombosis and has been a target for much research in the development of anti-platelet or anti-adhesive agents. One strategy has been to utilize RGD peptides as anti-platelet or anti-adhesive agents. It is well known that small synthetic peptides that contain the tripeptide Arg-Gly-Asp (RGD) sequence or natural RGD-containing small proteins derived from snake venoms, collectively termed disintegrins, inhibit the binding of fibrinogen to the GPIIb/IIIa receptor and abolish aggregation. However, RGD peptides are generally not suitable for *in vivo* use because of their short half life in the body. It would be desirable to be able to develop therapeutic agents which retain the positive properties of RGD-containing peptides for longer periods of time than is normally currently the case.

SUMMARY OF THE INVENTION

This invention relates to novel chemically reactive derivatives of RGD-containing peptides which can react with available functionalities on mobile blood proteins to form covalent linkages, and in which the resulting covalently bound conjugates have RGD peptide activity.

As compared with RGD peptide drugs the conjugated molecules have extended lifetimes in the bloodstream and are, therefore, capable of maintaining RGD peptide activity for extended periods of time as compared to the unconjugated parent drug, and provide such activity with reduced centrally mediated side effects.

The invention also includes the selective labeling of mobile endogenous proteins such as serum albumin and IgG with RGD-maleimide peptides. In addition, the invention includes the non-specific labeling of mobile blood proteins with NHS-RGD peptides and sulfo-NHS peptides. NHS is N-hydroxysuccinimide. Sulfo-NHS is N-hydroxysulfosuccinimide.

The invention further includes the conjugates of the RGD

peptide derivatives with mobile blood components and methods for providing RGD peptide activity *in vivo* comprising administering to a mammalian host the novel RGD peptide derivatives or their conjugates.

5 This invention also relates to use of antibodies to locate and bind to such conjugates, for instance, to remove undesirable excesses of them from the host's blood stream. The invention also relates to the use of antibodies to detect levels of RGD peptides in blood.

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BRIEF DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1 is an acetylated RGD peptide.

SEQ ID NO:2 is an acetylated maleimide RGD peptide.

SEQ ID NO:3 is an acetylated NHS RGD peptide.

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DETAILED DESCRIPTION OF THE INVENTION

To ensure a complete understanding of the invention, the following definitions are provided:

20 **RGD Peptides:** RGD peptides are peptides comprised of amino acids that include the sequence R-G-D where R is arginine, G is glycine and D is aspartic acid.

Reactive Entities: Reactive entities are entities capable of
25 forming a covalent bond. Such reactive agents are coupled or bonded to an RGD peptide of interest. Reactive entities will generally be stable in an aqueous environment and will usually be carboxy, phosphoryl, or convenient acyl group, either as an ester or a mixed anhydride, or an imidate, thereby capable of forming a
30 covalent bond with functionalities such as an amino group, a hydroxy or a thiol at the target site on mobile blood components. For the most part, the esters will involve phenolic compounds, or b

thiol esters, alkyl esters, phosphate esters, or the like.

Functionalities: Functionalities are groups on blood components including mobile proteins or RGD peptide derivatives to which reactive entities react to form covalent bonds. Functionalities include hydroxyl groups for bonding to ester reactive entities; thiol groups for bonding to imidates and thioester groups; amino groups for bonding to carboxy, phosphoryl or acyl groups on reactive entities and carboxyl groups for bonding to amino groups.

Mobile Blood Proteins: Mobile blood proteins are soluble blood proteins. These blood proteins are not membrane-associated and are present in the blood for extended periods of time and are present in a minimum concentration of at least 0.1 µg/ml. Mobile blood proteins include serum albumin, transferrin, ferritin and immunoglobulins such as IgM and IgG. The half-life of mobile blood proteins is at least about 12 hours.

Protective Groups: Protective groups are chemical moieties utilized to protect RGD peptide derivatives from reacting with themselves. Various protective groups are disclosed in U.S. 5,493,007 which is hereby incorporated by reference. Such protective groups include acetyl, fluorenylmethyloxycarbonyl (FMOC), BOC, CBZ, and the like.

Linking Groups: Linking groups are chemical moieties that link or connect reactive entities to RGD peptides. Linking groups may comprise one or more alkyl groups, alkoxy group, alkenyl group, alkynyl group or amino group substituted by alkyl groups, cycloalkyl group, polycyclic group, aryl groups, polyaryl groups, substituted aryl groups, heterocyclic groups, and substituted heterocyclic groups.

Taking into account these definitions, this invention relates to compositions which are derivatives of RGD peptides which can react with the available reactive functionalities on blood components via covalent linkages. The invention also relates to such derivatives, such combinations with blood components, and methods for their use. These methods include extending the effective of therapeutic life of the native RGD peptide in question as compared to administration of the RGD peptide per se to a patient. The RGD peptide derivative is of a type designated as a DAC (drug affinity complex) which comprises the RGD peptide molecule and a linking group together with a chemically reactive group capable of reaction with a reactive functionality of a mobile blood protein. By reaction with the blood component or protein the RGD peptide derivative or DAC may be delivered via the blood to appropriate sites or receptors of the patient such as platelet glycoprotein IIb/IIIa or other integrin receptors such as $\alpha_v\beta_3$.

To form covalent bonds with the functional group on the protein, one may use as a chemically reactive group a wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable at the levels required. While a number of different hydroxyl groups may be employed in these linking agents, the most convenient would be N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS) and maleimidopropionic acid (MPA). In the preferred embodiments of this invention, the functional group on this protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as (GMBA or MPA). GMBA stands for gamma-maleimide-butyrylamide.

The RGD peptide for conjugation to cells, erythrocytes or mobile endogenous proteins in accordance with the present invention includes a sequence of amino acids, preferably naturally occurring

L-amino acids and glycine, having the following formula:



In this formula, R_1 and R_2 represent an amino acid or a sequence of more than one amino acid or a derivatized or chemically modified amino acid or more than one derivatized or chemically modified amino acids.

In a specific embodiment, R_1 represents $XY(Z)_n$, in which X, Y and Z independently represent an amino acid; and n represents 0 or 1; R_2 represents OH or NH_2 ; or any amino acid; or a sequence of more than one amino acid or a derivatized or chemically modified amino acid. In a specific embodiment, R_2 represents an amino acid other than serine, threonine or cysteine or the amide thereof wherein the amino acid is rendered a carboxyamide. In another specific embodiment, R_2 is more than one amino acid, the first amino acid in the sequence, which is attached to aspartic acid, being other than serine, threonine or cysteine, or the amide of any free carboxyl groups wherein R_2 includes a derivatized or chemically modified amino acid.

In a preferred embodiment, R_2 includes a linking group having a chemically reactive group which covalently bonds to reactive functionalities or proteins and R_1 includes a protective group to prevent the chemically reactive group of R_2 from reacting with R_1 . In another embodiment, R_1 includes a linking group having a chemically reactive group which covalently bonds to reactive functionalities on proteins and R_2 includes a protective group to prevent the chemically reactive group of R_1 from reacting with R_2 .

In yet another embodiment, both R_1 and R_2 include a linking group having a chemically reactive entity which covalently bonds to functionalities on mobile proteins. In this embodiment, the linking groups may be similar or different.

In the RGD peptide of this invention, I, R_1 and R_2 may include any amino acid or sequence thereof. The amino acids are preferably

naturally occurring. The most common naturally-occurring amino acids are shown in Table I:

TABLE I NATURAL AMINO ACIDS AND THEIR ABBREVIATIONS		
Name	3-Letter Abbreviation	1-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Try	W
Tyrosine	Tyr	Y
Valine	Val	V

However, R₁ and R₂ in the RGD peptide of this invention are not limited to the 20 natural-amino acids. In other embodiments, R₁ and R₂ can be D-amino acids, non-classical amino acids or cyclic peptides or peptidomimetics (chemical peptide analogs). Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, designer amino acids such as β -methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general.

Furthermore, the Arg and/or Asp in the RGD sequence can be the D (dextrarotary) or L (levorotary) amino acid.

When R_1 and/or R_2 are a sequence of amino acids, there is no necessary limitation on the number of amino acids in the sequence(s). Accordingly, the polypeptide for conjugation to mobile blood proteins can be any size, and encompasses what might
5 otherwise be called an oligopeptide, a protein, an organic molecule or a polymer such as polyethylene glycol. Preferably, the polypeptide will have no more than about 1,000 amino acids.

The polypeptide may be prepared by methods that are known in the art. For example, in brief, solid phase peptide synthesis
10 consists of coupling the carboxyl group of the C-terminal amino acid to a resin and successively adding N-alpha protected amino acids. The protecting groups may be any known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. The
15 coupling of amino acids to appropriate resins is described by Rivier et al., U.S. Pat. No. 4,244,946. Such solid phase syntheses have been described, for example, by Merrifield, 1964, *J. Am. Chem. Soc.* 85:2149; Vale et al., 1981, *Science* 213:1394-1397; Marki et al., 1981, *J. Am. Chem. Soc.* 103:3178 and in U.S. Pat. Nos.
20 4,305,872 and 4,316,891.

Derivatives of RGD peptides and their analogs which can conjugate with proteins and other mobile blood proteins are prepared as is known in the art by the use of linking groups having chemically reactive groups which covalently bond to reactive functionalities on
25 proteins.

Specific Labeling.

Preferably, the RGD peptides of this invention are designed to specifically react with thiol groups on mobile blood proteins. Such reaction is preferably established by covalent bonding of a maleimide link (e.g. prepared from GMBS, MPA or other maleimides) to a thiol
30 group on a mobile blood protein such as albumin or IgG.

Under certain circumstances, specific labeling with maleimides

offers several advantages over non-specific labeling of mobile proteins with groups such as NHS and sulfo-NHS. Thiol groups are less abundant *in vivo* than amino groups. Therefore, the maleimide RGD peptide derivatives of this invention will covalently bond to fewer proteins. For example, in albumin (the most abundant blood protein) there is only a single thiol group. Thus, RGD-maleimide-albumin conjugates will tend to comprise approximately a 1:1 ratio of RGD peptide derivatives to albumin. In addition to albumin, IgG molecules (class II) also have free thiols. Since IgG molecules and serum albumin make up the majority of the soluble protein in blood they also make up the majority of the free thiol groups in blood that are available (to covalently bond to) maleimide-RGD peptide derivatives.

Through controlled administration of maleimide-RGD peptide *in vivo*, one can control the specific labeling of albumin and IgG *in vivo*. In typical administrations, 80-90% of the administered maleimide-RGD peptide will label albumin and 10-20% will label IgG. Trace labeling of free thiols such as glutathione will also occur. Such specific labeling is preferred for *in vivo* use as it permits an accurate calculation of the estimated half-life of the administered agent.

In addition to providing controlled specific *in vivo* labeling, maleimide-RGD peptides can provide specific labeling of serum albumin and IgG *ex vivo*. Such *ex vivo* labeling involves the addition of maleimide-RGD to blood, serum or saline solution containing serum albumin. Once modified *ex vivo* with RGD-Mal, the blood, serum or saline solution can be readministered to the blood for *in vivo* treatment.

In contrast to NHS-peptides, maleimide-RGD peptides are generally quite stable in the presence of aqueous solutions and in the presence of free amines. Since maleimide RGD derivatives will only react with free thiols, protective groups are generally not necessary to prevent the maleimide-RGD peptide derivative from reacting with

itself. In addition, the increased stability of the peptide permits the use of further purification steps such as HPLC to prepare highly purified products suitable for *in vivo* use. Lastly, the increased chemical stability provides a product with a longer shelf life.

5 **Non-Specific Labeling.**

Bonds to amino groups will also be employed, particularly with the formation of amide bonds for non-specific labeling. To form such bonds, one may use as a chemically reactive group a wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable at the levels required. While a number of different hydroxyl groups may be employed in these linking agents, the most convenient would be N-hydroxysuccinimide (NHS) and N-hydroxy-sulfosuccinimide (sulfo-NHS).

Other linking agents which may be utilized are described in U.S. Patent 5,612,034, which is hereby incorporated herein.

The various sites with which the chemically reactive group of the subject non-specific RGD peptide derivatives may react *in vivo* include cells, particularly red blood cells (erythrocytes) and platelets, and proteins, such as immunoglobulins, including IgG and IgM, serum albumin, ferritin, steroid binding proteins, transferrin, thyroxin binding protein, α -2-macroglobulin, and the like. Those receptors with which the derivatized RGD peptides react, which are not long-lived, will generally be eliminated from the human host within about three days. The proteins indicated above (including the proteins of the cells) will remain at least three days, and may remain five days or more (usually not exceeding 60 days, more usually not exceeding 30 days) particularly as to the half life, based on the concentration in the blood.

For the most part, reaction will be with mobile components in the blood, particularly blood proteins and cells, more particularly blood proteins and erythrocytes. By "mobile" is intended that the component does not have a fixed situs for any extended period of

time, generally not exceeding 5, more usually one minute, although some of the blood component may be relatively stationary for extended periods of time. Initially, there will be a relatively heterogeneous population of functionalized proteins and cells.

5 However, for the most part, the population within a few days will vary substantially from the initial population, depending upon the half-life of the functionalized proteins in the blood stream. Therefore, usually within about three days or more, IgG will become the predominant functionalized protein in the blood stream.

10 Usually, by day 5 post-administration, IgG, serum albumin and erythrocytes will be at least about 60 mole %, usually at least about 75 mole %, of the conjugated components in blood, with IgG, IgM (to a substantially lesser extent) and serum albumin being at least about 50 mole %, usually at least about 75 mole %, more usually at
15 least about 80 mole %, of the non-cellular conjugated components.

Preferably, the RGD peptide derivative is conjugated to albumin.

The desired conjugates of non-specific RGD peptides to blood components may be prepared *in vivo* by administration of the RGD
20 peptide derivatives to the patient, which may be a human or other mammal. The administration may be done in the form of a bolus or introduced slowly over time by infusion using metered flow or the like.

If desired, the subject conjugates may also be prepared *ex vivo*
25 by combining blood with derivatized RGD peptides of the present invention, allowing covalent bonding of the derivatized RGD peptides to reactive functionalities on blood components and then returning or administering the conjugated blood to the host. Moreover, the above may also be accomplished by first purifying an individual blood
30 component or limited number of components, such as red blood cells, immunoglobulins, serum albumin, or the like, and combining the component or components *ex vivo* with the chemically reactive

RGD peptide derivatives. The functionalized blood or blood component may then be returned to the host to provide *in vivo* the subject therapeutically effective conjugates. The blood also may be treated to prevent coagulation during handling *ex vivo*.

5 The RGD peptide derivatives will be administered in a physiologically acceptable medium, e.g. deionized water, phosphate buffered saline (PBS), saline, aqueous ethanol or other alcohol, plasma, proteinaceous solutions, mannitol, aqueous glucose, alcohol, vegetable oil, or the like. Other additives which may be included
10 include buffers, where the media are generally buffered at a pH in the range of about 5 to 10, where the buffer will generally range in concentration from about 50 to 250 mM, salt, where the concentration of salt will generally range from about 5 to 500 mM, physiologically acceptable stabilizers, and the like. The compositions
15 may be lyophilized for convenient storage and transport.

 The subject RGD peptide derivatives will for the most part be administered parenterally, such as intravascularly (IV), intraarterially (IA), intramuscularly (IM), subcutaneously (SC), or the like. Administration may in appropriate situations be by transfusion. In
20 some instances, where reaction of the functional group is relatively slow, administration may be oral, nasal, rectal, transdermal or aerosol, where the nature of the conjugate allows for transfer to the vascular system. Usually a single injection will be employed although more than one injection may be used, if desired. The RGD
25 peptide derivatives may be administered by any convenient means, including syringe, trocar, catheter, or the like. The particular manner of administration will vary depending upon the amount to be administered, whether a single bolus or continuous administration, or the like. Preferably, the administration will be intravascularly, where
30 the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. Other routes may find use where the administration is

coupled with slow release techniques or a protective matrix. The intent is that the RGD peptide, analog or derivative be effectively distributed in the blood, so as to be able to react with the blood components. The concentration of the conjugate will vary widely, generally ranging from about 1 pg/ml to 50 mg/ml. The total administered intravascularly will generally be in the range of about 0.1 mg/ml to about 10 mg/ml, more usually about 1 mg/ml to about 5 mg/ml.

By bonding to long-lived components of the blood, such as immunoglobulin, serum albumin, red blood cells and platelets, a number of advantages ensue. The activity of the RGD peptide compound is extended for days to weeks. Only one administration need be given during this period of time. Greater specificity can be achieved, since the active compound will be primarily bound to large molecules, where it is less likely to be taken up intracellularly to interfere with other physiological processes.

The blood of the mammalian host may be monitored for the presence of the RGD peptide compound one or more times. By taking a portion or sample of the blood of the host, one may determine whether the RGD peptide has become bound to the long-lived blood components in sufficient amount to be therapeutically active and, thereafter, the level of RGD peptide compound in the blood. If desired, one may also determine to which of the blood components the RGD peptide or RGD peptide derivative molecule is bound. This is particularly important when using non-specific RGD peptides. For specific maleimide-RGD peptides, it is much simpler to calculate the half life of serum albumin and IgG.

Thus, this invention relates to such conjugates of RGD peptides, RGD peptide analogs and their derivatives with blood components, particularly blood proteins such as albumin, as well as methods of administering them to human and other mammal patients.

Another aspect of this invention relates to methods for determining the concentration of the RGD peptides and/or analogs, or their derivatives and conjugates in biological samples (such as blood) using antibodies specific to the RGD peptides or RGD peptide analogs or their derivatives and conjugates, and to the use of such antibodies as a treatment for toxicity potentially associated with such RGD peptides, analogs, and/or their derivatives or conjugates. This is advantageous because the increased stability and life of the RGD peptides *in vivo* in the patient might lead to novel problems during treatment, including increased possibility for toxicity. The use of anti-therapeutic agent antibodies, either monoclonal or polyclonal, having specificity for a particular RGD peptide, RGD peptide analog or derivative thereof, can assist in mediating any such problem. The antibody may be generated or derived from a host immunized with the particular RGD peptide, analog or derivative thereof, or with an immunogenic fragment of the agent, or a synthesized immunogen corresponding to an antigenic determinant of the agent. Preferred antibodies will have high specificity and affinity for native, derivatized and conjugated forms of the RGD peptide or RGD peptide analog. Such antibodies can also be labeled with enzymes, fluorochromes, or radiolables.

Antibodies specific for derivatized RGD peptides may be produced by using purified RGD peptides for the induction of derivatized RGD peptide-specific antibodies. By induction of antibodies, it is intended not only the stimulation of an immune response by injection into animals, but analogous steps in the production of synthetic antibodies or other specific binding molecules such as screening of recombinant immunoglobulin libraries. Both monoclonal and polyclonal antibodies can be produced by procedures well known in the art.

The anti-therapeutic agent antibodies may be used to treat toxicity induced by administration of the RGD peptide, analog or

derivative thereof, and may be used *ex vivo* or *in vivo*. *Ex vivo* methods would include immuno-dialysis treatment for toxicity employing anti-therapeutic agent antibodies fixed to solid supports. *In vivo* methods include administration of anti-therapeutic agent antibodies in amounts effective to induce clearance of antibody-agent complexes.

The antibodies may be used to remove the RGD peptides, analogs or derivatives thereof, and conjugates thereof, from a patient's blood *ex vivo* by contacting the blood with the antibodies under sterile conditions. For example, the antibodies can be fixed or otherwise immobilized on a column matrix and the patient's blood can be removed from the patient and passed over the matrix. The RGD peptides or RGD peptide analogs, derivatives or conjugates, will bind to the antibodies and the blood containing a low concentration of the RGD peptide, analog, derivative or conjugate, then may be returned to the patient's circulatory system. The amount of RGD peptide compound removed can be controlled by adjusting the pressure and flow rate. Preferential removal of the RGD peptides, analogs, derivatives and conjugates from the plasma component of a patient's blood can be effected, for example, by the use of a semipermeable membrane, or by otherwise first separating the plasma component from the cellular component by ways known in the art prior to passing the plasma component over a matrix containing the anti-therapeutic antibodies. Alternatively the preferential removal of RGD peptide-conjugated blood cells, including red blood cells, can be effected by collecting and concentrating the blood cells in the patient's blood and contacting those cells with fixed anti-therapeutic antibodies to the exclusion of the serum component of the patient's blood.

The anti-therapeutic antibodies can be administered *in vivo*, parenterally, to a patient that has received the RGD peptide, analogs, derivatives or conjugates for treatment. The antibodies will bind the

RGD peptide compounds and conjugates. Once bound the RGD peptide activity will be hindered if not completely blocked thereby reducing the biologically effective concentration of RGD peptide compound in the patient's bloodstream and minimizing harmful side effects. In addition, the bound antibody-RGD peptide complex will facilitate clearance of the RGD peptide compounds and conjugates from the patient's blood stream.

The derivatives and conjugates of the RGD peptides and their analogs may be used in several different ways and to achieve several different ends. As mentioned above, these materials may be used in place of typical RGD peptide drugs as an anti-platelet or anti-adhesive agent. As compared with RGD peptide drugs currently available, the materials of this invention can reduce clot formation with less side effects and are available for reducing clot formation for a substantially longer time than conventionally administered RGD peptide drugs. In addition, the derivatized RGD peptides of this invention may be utilized (in accordance with U.S. Patent Numbers 5,443,827; 5,439,88 and 5,433,940 and PCT application number WO/97/01093 which are hereby incorporated by reference) in conjunction with various other anti-platelet or anti-adhesive therapies. Such therapies include the use of aspirin, hirudin, argatroban and argatroban derivatives, streptokinase, tissue plasminogen activator and the like.

The invention is further illustrated by the following examples.

Example 1

General

Products from the following examples were purified by preparative reversed phase HPLC using a Varian (Rainin) preparative binary HPLC system: gradient elution of 5-60% B (0.045% TFA in H₂O (A) and 0.045% TFA in CH₃CN (B)) at 9.5 mL/min using a Dynamax C₁₈, 60Å, 8 µm, 21 mm x 25 cm column equipped with a

Dynamax C₁₈, 60Å, 8 µm guard module and a UV detector (Varian Dynamax UVD II) detecting at λ214 and 254 nm. Analytical HPLC were performed using a Varian (Rainin) binary HPLC system: gradient elution of 5-60% B (0.045% TFA in H₂O (A) and 0.045% TFA in CH₃CN (B)) at 0.5 mL/min using a Dynamax C₁₈, 60Å, 8 µm, 4.6 mm x 25 cm column equipped with a Dynamax C₁₈, 60Å, 8 µm guard module and an UV detector (Varian Dynamax UVD II) detecting at λ214 and 254 nm. Mass spectrometry was performed on a PE Sciex API III electro-spray Biomolecular Mass Analyzer.

10

Example 2

Synthesis of Ac-RIARGDFPDDRK-NH₂ • 4 TFA

Syntheses of Ac-RIARGDFPDDRK-NH₂ peptide was performed on an ABI 433A Peptide Synthesizer using 510 mg of 0.49 mmol/g of Fmoc protected Rink Amide MBHA resin (NovaBiochem), 4 eq. of Fmoc protected amino acids, 4 eq. of a 0.45 M O-benzotriazol-1-yl-*N,N,N',N'*-tetramethyl-uronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) in *N,N*-dimethylformamide solution and activation with 4 eq. of 2 M *N,N*-diisopropylethylamine (DIEA) in 1-methyl-2-pyrrolidinone (NMP), and piperidine deprotection of Fmoc groups. Upon completion of the sequence, the resin was dried to afford 990 mg of a tan resin (91%).

20

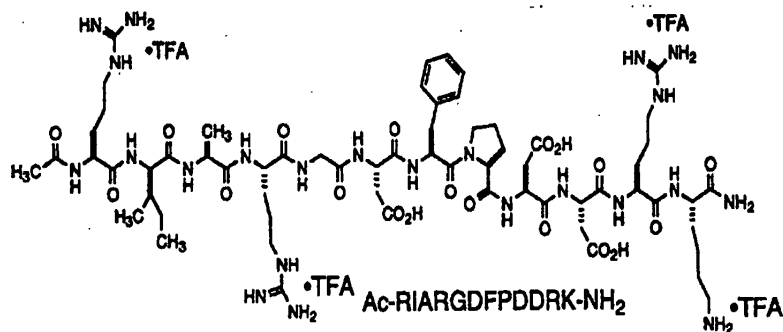
The peptide was removed from the resin by shaking 609 mg of Ac-RIARGDFPDDRK-MBHA-Resin with two 5 mL portions of a cleavage cocktail (comprised of: 10 mL of trifluoroacetic acid (TFA); 0.75 g of phenol; 0.25 g of thioanisole; 0.5 mL of ethanedithiol (EDT); and 0.5 mL of water) for 2 h each. The filtrates were each collected and combined and combined with the filtrates from washing the resin with 5 mL of TFA and 5 mL of CH₂Cl₂. The filtrates were then concentrated to approximately 10 mL and the product was precipitated out by the addition of 40 mL of dry-ice cold Et₂O. The resulting precipitate was collected by centrifugation and

25

30

re-suspended in of 40 mL of dry-ice cold Et₂O, centrifuged and process repeated to afford 163 mg of the crude peptide as a white solid (0.084 mmol, 60%). Analytical HPLC showed purity to be approximately 72%

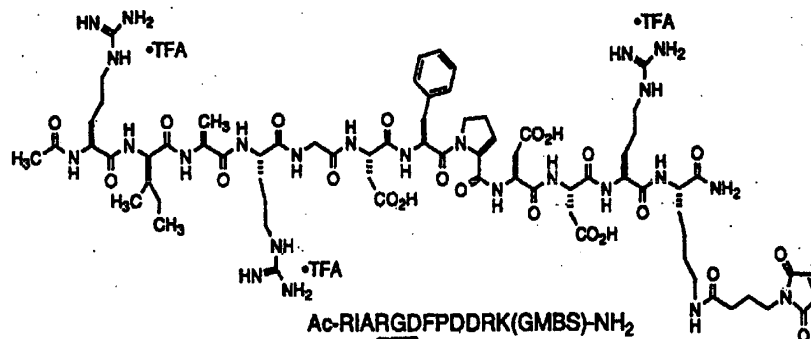
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Example 3

Synthesis of Ac-RIARGDFPDDRK(GMBA)-NH₂ • 3 TFA

In a 50-mL centrifuge tube, 163 mg of crude peptide (0.084 mmol) Ac-RIARGDFPDDRK-NH₂ (Example 2) was dissolved in 1.2 mL of DMF. To this solution was added a slightly yellow solution of 25.9 mg of *N*-[γ-maleimidobutyryloxy]succinimide ester (GMBS) (0.092 mmol), 117 μL of TEA (85.0 mg, 0.84 mmol) in 300 μL of DMF. The reaction was vortexed for 15 sec and allowed to stand at RT for 45 min. The reaction was quenched by the addition of 136 μL of TFA (1.68 mmol), vortexed for 30 sec and product oiled out by the addition of 30 mL of dry-ice cold Et₂O. The Et₂O was removed and oil washed with 30 mL more of dry-ice cold Et₂O, then dissolved in 2 mL of 0.045% TFA in CH₃CN and 8 mL 0.045% TFA in water, purified by preparative HPLC and desired fractions collected and lyophilized to afford 83.1 mg of product as a pale yellow solid (0.042 mmol, 50%). Analytical HPLC indicated product to be >80% pure with R_t = 30.59 min (product) and 31.37 min (impurity). ESI-MS *m/z* for C₇₁H₁₁₀N₂₄O₂₂ (MH⁺), calculated 1650.8, found MH³⁺ 551.5, MH²⁺ 826.3.



Example 4

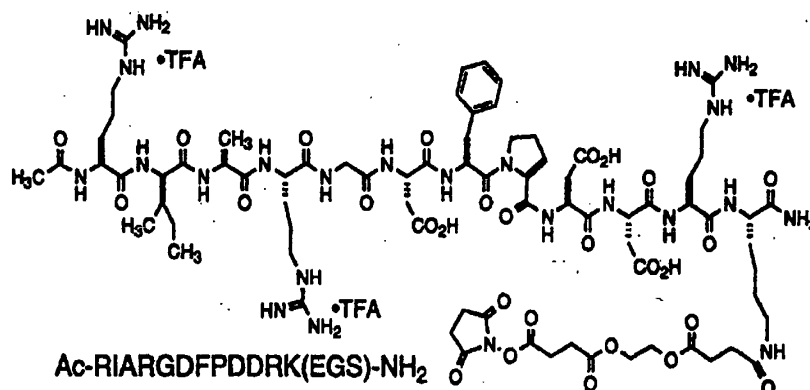
5 Synthesis of Ac-RIARGDFPDDRK(EGS)-NH₂ • 3 TFA

In a 15-mL centrifuge tube, 46.2 mg of ethylene glycol-bis(succinimidylsuccinate) (0.071 mmol) and 8.50 μ L of triethylamine (0.061 mmol) was dissolved in 500 μ L of DMF. To this vortexing solution was added dropwise over 30 sec a solution of

10 12.2 mg of Ac-RIARGDFPDDRK-NH₂ • 4 TFA (0.006 mmol) (Example 2) in 100 μ L of DMF and following addition the reaction was allowed to stand at RT for 1.5 h. To this was added 9.88 μ L of TFA (0.122 mmol), vortexed and product precipitated out by the addition of 15-mL of dry-ice cold Et₂O. The precipitate was collected

15 by centrifugation and solid taken up in 1 mL of 0.045% TFA in CH₃CN and 1 mL 0.045% TFA in water and purified by preparative HPLC. The desired fractions were collected and lyophilized to afford 6.80 mg of product as a white solid (0.003 mmol, 50%). Analytical HPLC indicated product to be >70% pure with R_t = 38.17 min

20 (product) and 37.21 min (hydrolysis product) ESI-MS *m/z* for C₇₇H₁₁₉N₂₄O₂₈ (MH⁺), calcd 1827.9, found MH²⁺ 914.8. Hydrolysis product ESI-MS *m/z* for C₇₃H₁₁₆N₂₃O₂₆ (MH⁺), calcd 1731.9, found MH²⁺ 866.2.



Example 5

Synthesis of MPA-AEA₃RIARGDFPDDRK-NH₂

5 Using automated peptide synthesis, the following protected amino acids and maleimide could be sequentially added to Ring Amide MBHA resin: Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Pro(Boc)-OH, Fmoc-Phe-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH, Fmoc-Ile-OH, Fmoc-Arg(Pbf)-OH, Fmoc-AEA-OH, Fmoc-AEA-OH, Fmoc-AEA-OH, and MPA. The target RGD peptide analog is then removed from the resin; the product is isolated by precipitation and purified by preparative HPLC to afford the desired product.

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Example 6

Anchoring of activated RGD peptides to human plasma proteins.

Activated RGD peptides were anchored to human plasma proteins by the following procedures.

20

I-Description of peptides.

The following RGD peptides were utilized:

- Triflavin-like peptide : acetylated RIARGDFPDDRK (Example 2)
- GMBS activated peptide : acetylated RIARGDFPDDRK-Mal (Example 3)

- EGS activated peptide : acetylated RIARGDFPDDRK-NHS
(Example 4)

For each peptide, a 40 mM stock-solution was prepared in DMSO.

5

II-Methods.

A. Preparation of Platelet Poor Plasma (PPP) and Anchoring to Human Plasma Proteins.

10 Venous blood was withdrawn from normal donor and placed in citrated tubes. Blood samples were centrifuged for 10 minutes at 2500 g in order to obtain platelet-poor-plasma (PPP).

PPP was mixed with RGD peptides at 100 μ M final concentration. After 1 hour-incubation at room temperature with
15 gentle stirring, plasma samples were extensively dialyzed against PBS containing 14 mM sodium citrate, at 4°C. Aliquots before dialysis were kept at +4°C and they were used for Western-blot analysis (see section C below). Dialyzed aliquots are used in functional assay (see section B below)

20

B. ADP-induced Platelet Aggregation.

1-Preparation of platelet-rich-plasma (PRP).

25 Venous blood was withdrawn on 0.129 M sodium citrate from normal donors who had not ingested any drugs (i.e. aspirin) for at least 10 days prior to blood donation. Blood was centrifuged at 250 g for 10 min. and the PRP (supernatant) was removed with plastic pipette. Platelet-poor-plasma (PPP) was obtained by spinning the red cell sediment at 2500 g for 10 min. Platelet concentration of PRP
30 was determined using Coulter T40 and adjusted to 280,000 to 340,000 plts/ μ l with PPP.

2-Aggregation on S rvibi aggregom t r.

Stirring was adjusted to 1100 rpm and the temperature was regulated at 37°C. The aggregometer was first calibrated with 280 μ l of donor PPP which corresponds to 100% of transmission. For tested samples, 180 μ l of PRP were mixed with 100 μ l of dialyzed labeled plasma (as prepared in section A, either pure or diluted with PPP) and incubated for 1 min. at 37°C. The 0% of transmission was calibrated with this mixture and 20 μ l of 50 μ M ADP (adenosine diphosphate) were added. Light transmission was recorded during 350-450 sec. Four parameters were reported: velocity (% / min), aggregation at 3 min (%), maximum aggregation (%), area at 4 min. (% * sec). Each point was done in duplicate except the 2.5 μ .

C. Aggregation Velocity.

1-Inhibition of aggregation velocity.

In this assay, only one concentration was tested (33.33 μ M). No IC50 was determined. Velocity is one of the parameters utilized to measure the effect of an antagonist on aggregation. Initial concentration of RGD peptides in plasma = 100 μ M. Final concentration in the assay = 33.33 μ M (if conjugation is effective at 100%). The results are shown in Table 2.

Table 2

Inhibition of velocity obtained in 3 different experiments:

activated peptide	Test #1	Test #2	Test #3	Mean
acRGD-Mal	70.9%	67.13%	66.62%	68.32%
acRGD-NHS	52.94%	47.31%	57.72%	52.65%

2-IC50 Calculations.

In this assay, PPP samples were studied at several concentrations in order to determine IC50. The IC50 for the various peptides were calculated:

IC50 AcRGD-Mal = 21.53 μ M

IC50 AcRGD-NHS = 27.61 μ M

IC50 AcRGD = 5.7 μ M

5

The IC50 calculations indicate a loss of potency about of 3.8 to 4.8 times after derivatization and conjugation to plasma proteins. However, the derivatized RGD peptide remains active when covalently attached to proteins.

10

D. Western-blot analysis.

Ten μ l of treated plasma diluted 1:20 in water (about 30 μ g of plasma proteins) were solubilized by incubation in the Laemmli buffer (10 mM Tris, 1 mM EDTA, 5% SDS, pH 6.8) and boiled for 3 minutes in a water bath. Proteins were separated by SDS-PAGE on a 8% polyacrylamide gel under non-reducing conditions according to the method described by Laemmli (1970). Plasma proteins were then transferred to nitrocellulose membrane (semi-dry transfer) for 1 hour at 2 mA/cm². After transfer, the nitrocellulose membrane was saturated with TBS (10 mM Tris, 150 mM NaCl, pH 7.4) containing 5% gelatin and 0.1% Tween20, for 2 hours at 37°C. After 3 washes with TBS-0.1% Tween20, the blot was incubated with a rabbit polyclonal serum anti-RGD (rbt30T3) diluted 1:5000 in TBS-1% gelatin-0.1% Tween20, for 1.5 hours at room temperature. After 3 washes as above, the blot was incubated with a peroxidase-labeled goat anti-rabbit IgG (Sigma, A0545) diluted 1:200,000 in TBS-1% gelatin-0.1% Tween20, for 1 hour at room temperature. After 3 washes, blot revelation was performed with the ECL reagent (Amersham) as described by the manufacturer.

30

The western blot analysis indicates that AcRGD-mal binds preferentially to albumin (band at 57 kDa) and then IgG whereas acRGD-NHS gives a more intense signal on IgG and fibrinogen (bands

of 193 kDa and above) but binds also albumin and additional proteins.

Example 7

5 Anchoring of activated RGD peptides to rabbit serum albumin.

I-Description of peptides.

The peptides are the same as those described in example 6.

10 II-Methods.

A. Anchoring to rabbit serum albumin.

Peptides were solubilized at 40 mM in DMSO and incubated with 300 μ l RSA (rabbit serum albumin, Sigma A9638) at 100 μ M (6.6 mg/ml) in PBS at peptide/RSA ratio of 5/1 (final concentration of peptide is 500 μ M). After a 30-minute incubation at room temperature, conjugates were filtrated on PD10 column (5 ml desalting column, Pharmacia) equilibrated in PBS, and recovered in a 2 ml fraction. Conjugates were concentrated by ultrafiltration on Microsep (cut-off 10kDa) and adjusted to a concentration of 5 mg/ml as RSA after protein dosage (BCA, Pierce).

B. ADP-induced platelet aggregation.

25 1-Preparation of platelet-rich-plasma (PRP).

Platelet-rich plasma (PRP) was isolated as described in Example 6.

2-Aggregation on Servibio aggregometer.

30 Stirring was adjusted to 1100 rpm and temperature was regulated at 37°C. The aggregometer was first calibrated with 250 μ l of donor PPP mixed with 30 μ l of PBS which corresponds to

100% of transmission. For tested samples, 250 μ l of PRP were mixed with 30 μ l of RSA-RGD conjugates or free peptide diluted in PBS and incubated for 1 min. at 37°C. The 0% of transmission was calibrated with this mixture and 20 μ l of 50 μ M ADP (adenosine diphosphate, 3.3 μ M final concentration) were added. Light transmission was recorded during 350-450 sec. Four parameters were measured: velocity (% / min), aggregation at 3 min (%), maximum aggregation (%), area at 4 min. (% * sec). Each point was done two duplicates, except the 2.5 μ M RGD test which was done in one duplicate only. The results of the velocity measurements are presented in Table 3.

Table 3

Inhibition of velocity of clot formation by RGD peptides

RGD Molecule	% Inhibition of Velocity of Clot Formation
RGD (10 μ M)	79.26
RGD (5 μ M)	61.21
RGD (2.5 μ M)	44.66
RSA-NHS-RGD (500 μ g/ml)	60.99
RSA-Mal-RGD (500 μ g/ml)	24.47

15

These experiments were designed to compare the free RGD peptides to conjugated RGD peptides in terms of their abilities to inhibit the velocity of clot formation. The results show that free RGD peptides at 10 μ M concentration inhibit the velocity of clot formation by 79.26%. RSA-NHS-RGD at 500 μ g/ml inhibit the velocity of clot formation by 60.99% whereas RSA-Mal-RGD at 500 μ g/ml inhibit velocity by 24.47%. The data demonstrate that RGD peptides conjugated to RSA by maleimide or NHS retain activity.

Maleimide reacts with free thiols. Since RSA has only 1 free thiol and 50 to 60% of those thiols are capped and unavailable, Mal-RSA will generally react only with 40 to 50% of the RSA

5 molecules. In contrast, RGD-NHS will react anywhere there is a free amine. Since RSA has 52 free amines, RGD-NHS will bind with a higher copy number on RSA than will Mal-RGD. The increased copy number will result in higher % inhibition of clot formation as shown in Table 3.

C. Binding assay with immobilized GP IIb-IIIa.

1-Purification of platelet membrane glycoprotein GP IIb-IIIa complex.

10 Human platelets were obtained from outdated platelet concentrates that were stored at RT under constant stirring and used within 14 days of venipuncture. Briefly, platelet concentrates were centrifuged for 20 min at 2500 g at RT. Platelet pellets were washed 4 times with TBS (10mM Tris, 150mM NaCl) containing 15 mM EDTA, pH7, for 10 min at 2500 g. Platelets were lysed immediately or pellets were frozen at - 35°C until lysis. For lysis, 5 to 10 ml of lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1mM CaCl₂, 10⁻⁵ M Leupeptin, pH 7.4, cold) were added per ml of platelet pellet and incubated for 45 min in ice. Lysates were 20 centrifuged for 30 min at 30000 g at 4°C to remove insoluble material (cytoskeletal elements) and were frozen at - 80 °C until use.

Glycoprotein IIb-IIIa complexes were purified by three successive affinity chromatographies. The first purification step was performed on Concanavalin A-Sepharose 4B (Sigma, C-9017, lot 25 125H1071) which retains glycoprotein (gp) IIb, gpIIIa, thrombospondin and fibrinogen. Platelet lysates were thawed by immersion into a 37°C bath and centrifuged 30 min at 30,000 g at 4°C to remove insoluble material. In the meantime, Con A column was equilibrated with buffer A (10 mM Tris-HCl, 150 mM NaCl, 0,1 30 % Triton X-100, 0,05 % NaN₃, pH7,4. Lysate was applied on the column at a flow rate of 1 ml/min. at RT. After extensive washes with several bed volumes of buffer A, bound material was eluted

with buffer A containing 0.1 M α -D-m thyl-mannopyranoside (Sigma, M-6862, lot 118F-0632; MW 194.2) at a flow rate of 0.5 ml/min; fractions of 2 ml were collected and protein concentration was followed by optical density at 280nm. Eluted components were analyzed by SDS PAGE and Coomassie blue staining and fractions containing gpIIb and IIIa were pooled and stored at 4°C until next chromatography.

The second purification step consisted in removing thrombospondin component by chromatography on Heparin Agarose (Sigma, H-6508, lot 25H9508). The column was equilibrated with buffer A and gp IIb-IIIa containing fractions from the Con A column were applied at a flow rate of 1 ml/min. Flow through fractions containing gpIIb-IIIa and fibrinogen were stored at +4°C. Thrombospondin and degradation products binding to heparin-agarose were eluted with 0.5 M NaCl and column was used again for a new run.

Finally, the fibrinogen component was eliminated by chromatography on wheat germ agglutinin (WGA)-Sepharose (lectin from triticum vulgaris Sepharose 6 MB, Sigma, L-6257, lot 120H0318). After equilibration of the column in buffer A, Heparin flow through fraction was applied at a flow rate of 0.5 ml/min. The column was washed with buffer A extensively, fibrinogen being in the unretained fraction. Gp IIb and IIIa were eluted with buffer A containing 0.1 M N-acetyl-D-Glucosamine (Sigma, A-8625, lot 87F0329) at a flow rate of 0.5 ml/min; 1 ml fractions were collected. The purity of material was analyzed by SDS-PAGE and Coomassie blue staining and pure fractions were pooled, aliquoted and stored at -35°C until use.

30 2-Biotinylation of human fibrinogen.

Human fibrinogen (Sigma, F-3879, MW = 340 kDa) was dissolved at 1.5 mg/ml in 100 mM NaHCO_3 , 100 mM NaCl pH 8.2

and mixed with NHS-biotin (MW = 341.38) at 0.4 mM final concentration. The mixture was incubated for 30 minutes at room temperature on an orbital mixer. Uncoupled biotin was separated from biotinylated fibrinogen by gel filtration on a PD-10 column (Pharmacia) pre-equilibrated in 137 mM, 0.4 mM Na₂HPO₄, 2.7 mM KCl, 12 mM NaHCO₃ (pH 7.4). Fractions containing biotinylated fibrinogen were pooled and stored at 4 °C until use. The concentration of biotinylated fibrinogen used in the binding assay equals 570 µg/ml.

3-Fibrinogen binding assay.

Wells of a flat-bottom 96-well plate (Maxisorp, Nunc) were coated with 50 µl of purified IIb-IIIa (2 µg/ml in 20 mM Tris-HCl, 150 mM NaCl, 1 mM CaCl₂, 0.02% NaN₃ pH7.4) overnight at 4 C. Plate was flicked free of solution and saturated with 3% BSA in buffer described above, for 3 hours at room temperature (RT). After three washes with TBS, the plate was incubated with 50 µl/well of biotinylated fibrinogen diluted in TBS-1mM CaCl₂ for 1.5 hours at RT. For the inhibition test, 25 µl of inhibitor (free RGD or albumin-RGD conjugates) and immediately 25 µl of biotinylated fibrinogen were added. The plate was washed three times with TBS-0.05% Tween20 and incubated with peroxidase-labeled avidin (Sigma, A7419) diluted 1:50,000 in TBS-1 mM CaCl₂-1% BSA, for 1 hour at RT. After 3 washes as above, 100 µl of OPD (0.4 mg/ml in 50 mM Na₂HPO₄, pH5 with citrate 0.03% H₂O₂) were added per well. The reaction was stopped 15 minutes later with 1N sulfuric acid and OD were read at 492 nm on Multiskan MCC340.

The results are presented in Tables 4 and 5.

Table 4

Effect of RGD-RSA Conjugates on Fibrinogen Binding to GP IIb-IIIa

[RSA] μ M	% Inhibition of Clot Formation	
	RSA-NHS-RGD	RSA-Mal-RGD
7560	59.68	56.45
3780	50.40	42.17
1890	48.12	30.39
945	38.81	20.80
472	28.12	20.95
236	14.16	23.15

5

Table 5

Effect of RGD on fibrinogen binding to GP IIb-IIIa

[RGD/E]	% Inhibition of Clot Formation	
	RGD	RGE
35,000	74.09	-8.17
3500	68.52	-7.13
350	45.39	-13.57
35	14.35	-9.57
3.5	6.35	-7.30

10 The results in Table 5 show that free RGE has no effect on the % inhibition of clot formation but that free RGD has a strong inhibitory effect on the inhibition of clot formation. The IC₅₀ for free RGD is about 0.6 μ M.

15 The results in Table 4 show that RSA-RGD conjugates are able to inhibit binding of fibrinogen. In contrast, RSA alone has no effect on binding of fibrinogen. In this experiment, the IC₅₀ for RSA-NHS-RGD was 3.6 μ M whereas the IC₅₀ for RSA-Mal-RGD was 5.7 μ M. These results corroborate the aggregation data in Table 3.

WHAT IS CLAIMED IS:

1. A derivative of an RGD peptide, and analogs thereof, said derivative containing a reactive functional group which reacts with blood components *in vivo* to form stable covalent bonds.
- 5 2. An RGD peptide derivative according to claim 1 which reacts with blood proteins to form stable covalent bonds.
3. An RGD peptide derivative according to claim 1 in
10 which the reactive functional group is capable of reacting with an amino group, a carboxyl group, or a thiol group on the blood component.
4. An RGD peptide derivative according to claim 1
15 prepared by reaction of an RGD peptide or analog thereof with N-hydroxysuccinimide, N-hydroxysulfosuccinimide, maleimide-benzoyl-succinimide or gamma-maleimido-butyloxy succinimide.
5. A conjugate comprising an RGD peptide derivative, and
20 analogs thereof covalently bonded to a blood component *in vivo*.
6. A conjugate according to claim 5 in which the blood component is a blood protein.
7. A conjugate according to claim 5 in which the blood
25 protein is albumin or immunoglobulin.
8. A conjugate according to claim 5 in which the covalent
30 bond is formed between an amino, carboxyl or thiol group on said blood component and a reactive functional group contained in the RGD peptide derivative.

9. A blood composition comprising conjugate of blood components covalently bonded to an RGD peptide derivative which contains a reactive functional group which reacts with blood components, to form stable covalent bonds, said conjugates
5 resulting from the addition of a derivative of said RGD peptide or analog thereof to blood *in vivo*.

10. A conjugate according to claim 9 in which the blood component is a blood protein.

10

11. A conjugate according to claim 9 in which the blood protein is albumin or immunoglobulin.

12. A conjugate according to claim 9 in which the covalent
15 bond is formed between an amino, carboxyl or thiol group on said blood component and a reactive functional group contained in the RGD derivative.

13. A method of extending the life time of an RGD peptide
20 drug, said method comprising administering to the vascular system of a mammalian host a derivative of an analog comprising a reactive functional group which reacts with blood components to form stable covalent bonds, said reactive functional group being joined to said RGD or RGD analog, whereby said reactive functional group reacts
25 with at least one of protein and cells of the vascular system to produce at least one of modified protein and modified cells.

14. A method according to claim 13 in which the covalent bond is formed between the reactive functional group and an amino,
30 carboxyl or thiol group on the protein or cell.

15. A method according to claim 13 in which the blood component is a blood protein.

5 16. A method according to claim 16 in which the protein is albumin or immunoglobulin.

SEQUENCE LISTING

<110> EZRIN, ALAN M.
BRIDON, DOMINIQUE P.
HOLMES, DARREN L.
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THIBEDEAU, KAREN
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<120> NOVEL CONJUGATES OF RGD PEPTIDES AND ENDOGENOUS CARRIERS

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